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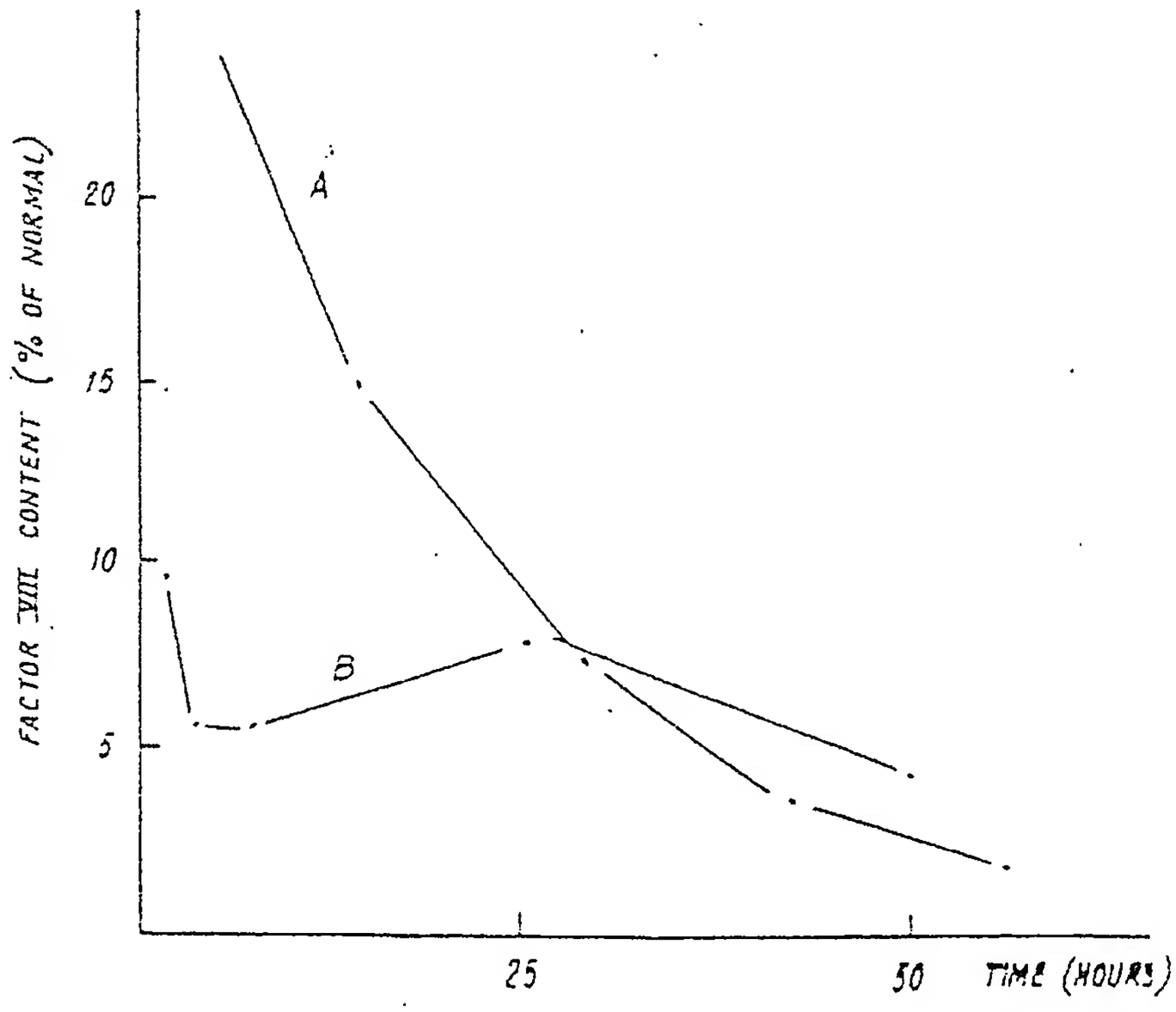

(57) Abstract

Pharmaceutical compositions containing liposomes formed from phospholipids in the presence of blood clotting factor VIII (anti-hemophilic factor) have been found to be active on oral administration by increasing the factor VIII blood plasma level. The invention relates to such pharmaceutical compositions and to methods for the preparation thereof.

Pharmaceutical composition and process for the preparation thereof.

The present invention relates to a pharmaceutical composition containing antihemophilic factor (factor VIII) as an active substance, as well as to a process for the preparation thereof.

Hemophilia A is a disease in which clotting factor VIII is present as an inactive genetic varation. In Von Willebrand's disease clotting factor VIII is present in the blood plasma in low concentrations. Both of the diseases are characterised by abnormal bleeding tendency. The bleedings may be life-threatening, but may also lead to serious invalidity, among others by hemarthrosis and muscular atrophy.

Therapy consists of administration of factor VIII. This can be effected via the intravenous route only, because factor VIII is very much susceptible to attack by proteolytic enzymes. Therefore, oral administration of factor VIII does not result in uptake thereof in the blood. Administration of factor VIII is used in case of bleedings and, prophylactically, before surgical operations or in situations of increased bleeding risk (for example in practising sports). The disadvantage of this way of administration is the necessity of vena puncture. Although there are patients capable of injecting themselves, the requirements of sterility and of technique of vena puncture are of such a nature that, in most cases, help in a medical centre is needed. Further, the necessary vena puncture is sometimes difficult, such as with obese patients or with patients having cicatricated veins due to repeated puncture. Therefore, oral treatment of hemophilia is highly desirable.

It was found that pharmaceutical compositions containing anti-hemophilic factor (factor VIII) as an active substance are effective on oral administration if

stearic acid, and oleic acid.

The invention also relates to a process for preparing a pharmaceutical composition containing anti-hemophilic factor (factor VIII) as an active substance. This process is characterised by the fact that phospholipid liposomes are formed in the presence of factor VIII. Generally, this process is carried out by suspending a phospholipid in an aqueous solution of factor VIII. This suspending operation may be effected with the aid of ultrasound but also, quite easily, by dissolving the phospholipid in a volatile solvent, such as ethanol, bringing the solution into a flask and concentrating the solution in the flask in such a way that the phospholipid is deposited as a thin layer on the inner wall of the flask. A milky suspension of liposomes will be formed by adding an aqueous solution of factor VIII and, after addition of some glass beads, swinging vigorously. The liposomes may be removed by centrifugation and, if desired, may be re-suspended in water or in a physiological salt solution. The liposome suspension may be processed further to form a liquid mixture for oral administration which may contain, for example, taste corrigentia. Also, the concentrated or diluted liposome suspension may be brought into soft gelatin capsules. It is also possible to prepare compositions which pass the stomach unchanged and become active only in the intestines.

Plasma fractions enriched with respect to factor VIII may be prepared in various ways. In this connection reference can be made to Vox Sanguinis 30 (1976) pages 1-22. In practice a plasma fraction enriched with respect to factor VIII may be obtained most readily by keeping plasma for some time at a low temperature. This results in the formation of a precipitate which may be removed by centrifugation. The precipitate is called cryoprecipitate and it is excellently suitable for use in the present compositions. Cryoprecipitate contains fibrinogen as well. Surprisingly it was found that a

waterjet pump. In this way the lipids remain as a thin layer on the inner wall of the flask. To one of the flasks 100 ml of an isotonic (0.9% by weight of NaCl) solution of an enriched factor VIII preparation (AHF-Konzentrat SRK (human); Zentral Laboratorium Blutspendedienst SRK Switzerland; 10 ml of lyophilised preparation contains about 230 U of factor VIII) are added, as well as some glass beads. The mixture is swung vigorously by hand until the lipid film is removed from the wall (5 to 10 minutes). The milky suspension obtained is centrifuged at 27.000 x g during 20 minutes which results in floating of the liposomes. The lower liquid layer is added to another flask and is shaken again in the presence of glass beads until the formation of the liposomes is complete. After centrifugation, the amount of non-trapped factor VIII present in the lower liquid layer, may be determined. The liposome fractions of both of the centrifugations are mixed. The mixture is suitable for oral administration as such or after dilution with physiological salt solution and/or addition of taste corrigentia.

In this process the amount of factor VIII entrapped was about 80%. The amount of fibrinogen entrapped was only 25% which means an enrichment with respect to factor VIII.

Factor VIII was essayed with the "one stage" method of Veldkamp (Thrombos. Diathes haemorrh. 19, 279 (1968)).

Results.

A typical experiment is illustrated in the attached drawing. At time 0 (fasting patient) 800 units of factor VIII were administered per os in the form of the composition obtained as described above. This results in an increase of the factor VIII level in the plasma to about 10% of the normal value. The plasma level remains above about 5% of the normal value during 50 hours. It is remarked in this connection that a factor VIII level of > 5% is regarded to be a status of light hemophilia, that is to say, a clinical picture in which spontaneous bleedings occur very rarely only. Thus, the low dosis

C L A I M S.

1. Pharmaceutical composition containing anti-hemophilic factor (factor VIII) as an active substance, characterised in that the composition is suitable for oral administration and that factor VIII is incorporated in liposomes formed from phospholipids.

2. Composition according to claim 1, characterised in that the liposomes have been formed from phospholipids and a nett charged lipid.

3. Composition according to claim 1 or 2, characterised in that the phospholipid is egg lecithin.

4. Composition according to claims 1 - 3, characterised in that the nett charged lipid is a phosphatidic acid.

5. Composition according to claim 4, characterised in that the phosphatidic acid is a natural phosphatidic acid having oleic acid, palmitic acid and stearic acid radicals as fatty acid constituents.

6. A process for preparing a pharmaceutical composition containing anti-hemophilic factor (factor VIII) as an active substance, characterised by forming phospholipid liposomes in the presence of factor VIII.

7. The process of claim 6 characterised by suspending a phospholipid in an aqueous solution of factor VIII.

8. The process of claim 6 or 7, characterised by suspending a phospholipid and a nett charged lipophilic substance in an aqueous solution of factor VIII.

9. The process of claims 6 - 8, characterised in that the phospholipid is egg lecithin.

10. The process of claims 6 - 8, characterised in that the nett charged lipophilic substance is a phosphatidic acid.

11. The process of claim 10, characterised in that the phosphatidic acid is a natural phosphatidic acid containing oleic acid, palmitic acid and stearic acid radicals as fatty acid constituents.

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According to International Patent Classification (IPC) or to both National Classification and IPC

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II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System [5]	Classification Symbols
Int.Cl.[3]	A 61 K 35/14; A 61 K 37/04; B 01 J 13/02 A 61 K 9/50; A 61 K 9/52

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched [6]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
	GB, A, 1502774, published March 1, 1978 see claims 1,10,11, National Research Development Corporation --	1-11
	AU, A, 53107/73, published September 12, 1974 see claims 1,10,11; page 4, line 23 to page 5, line 17, Walo Lenzinger --	1-11
	Unlisted Drugs, volume 18, no. 7, July 1966 page 617, "AHF", see the abstract corresponding to Chem. Week 98 (23): 66, June 4, 1966 ------	1-11

* Special categories of cited documents: [15]

"A" document defining the general state of the art

"E" earlier document but published on or after the international filing date

"L" document cited for special reason other than those referred to in the other categories

"O" document referring to an oral disclosure, use, exhibition or other means

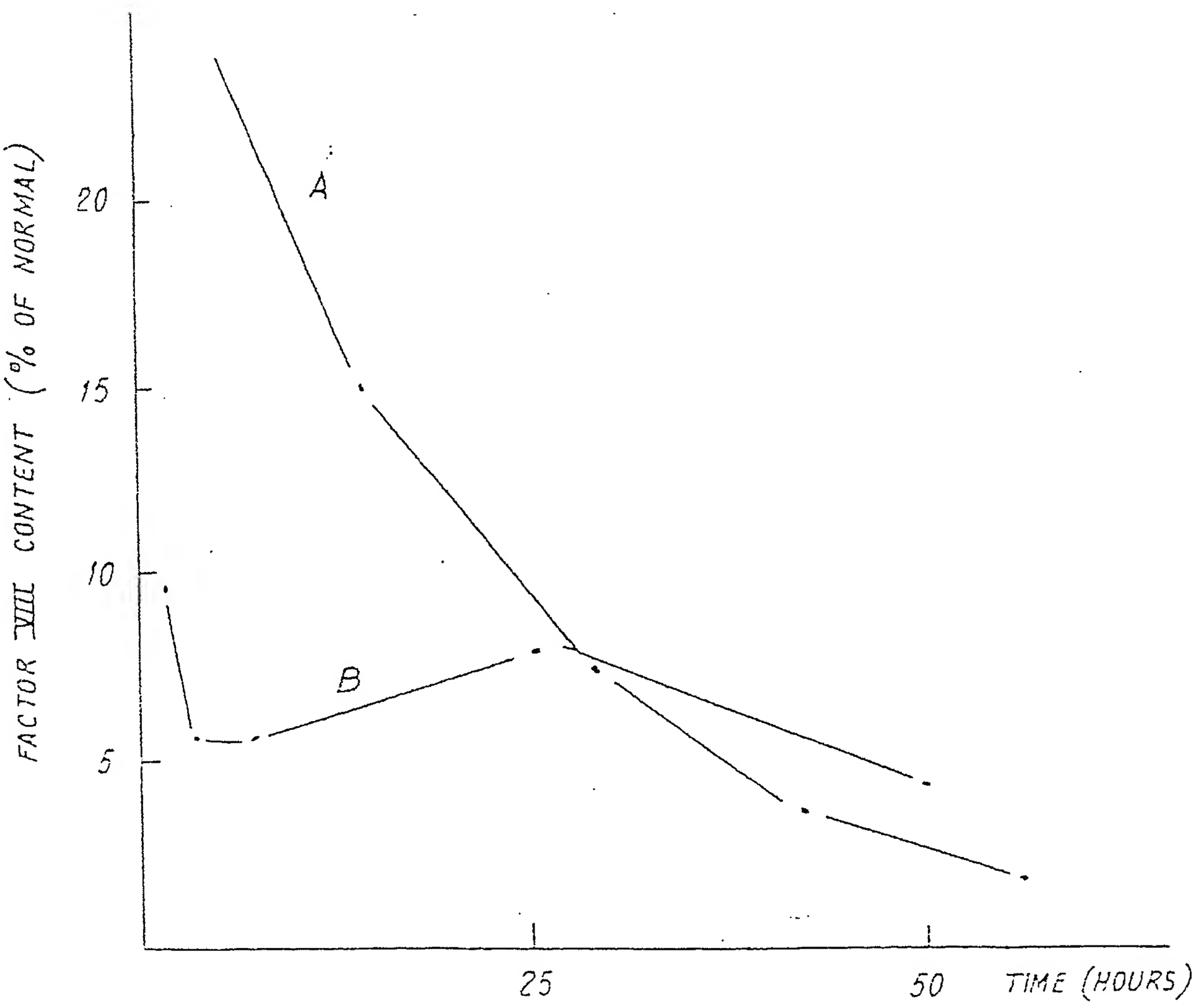
"P" document published prior to the international filing date but on or after the priority date claimed

"T" later document published on or after the international filing date or priority date and not in conflict with the application, but cited to understand the principle or theory underlying the invention

"X" document of particular relevance

IV. CERTIFICATION

Date of the Actual Completion of the International Search [3]	Date of Mailing of this International Search Report [3]
29th April 1980	13th May 1980
International Searching Authority [1]	**Signature of Authorized Officer [20]**
European Patent Office	G.L.M. Kruydenberg


FACTOR VIII CONTENT (% OF NORMAL)
20
15
10
5
A
B
25
50
TIME (HOURS)

Reverse transformation seemed to affect only tumour cells, since no effect was observed in the normal skin, mucosae, or other epithelial tissues. The selective character of this action explained the absence of toxicity of thioproline treatment.

The lack of activity of thioproline in experimental transplanted rodent tumours was not considered a setback. Serial transplantation might have caused important modifications of cell membranes which, while apparently not affecting tumour growth and response to cytotoxic drugs, might have impaired the response to the normal regulatory mechanisms of cell function that depend on membrane receptors.

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Requests for reprints should be addressed to A. B., Department of Oncology, Hospital General de Asturias, Oviedo, Spain.

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ORAL TREATMENT OF HÆMOPHILIA A BY GASTROINTESTINAL ABSORPTION OF FACTOR VIII ENTRAPPED IN LIPOSOMES

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Summary Factor-VIII-loaded liposomes were given orally to a patient with severe hæmophilia A. Plasma concentrations of factor VIII rose to therapeutically effective levels, that persisted for 50 hours.

INTRODUCTION

HÆMOPHILIA A is caused by a sex-linked congenital lack of functional coagulation factor VIII. Coagulation factor VIII is a plasma protein, as yet not well characterised but associated with a protein complex with a molecular weight of 2 000 000. In current treatment and prophylaxis of hæmophilia A, partly purified preparations of this protein are administered intravenously. We describe a preparation consisting of liposomes loaded with factor VIII that, when administered orally to a hæmophilic patient, produced a rise in plasma factor VIII procoagulant activity.

Liposomes are artificial structures that consist of multiple concentric bilayers of phospholipids.[1] Proteins may be entrapped in the interstices between the bilayers.[2] When liposomes are prepared in the presence of an aqueous solution of an enzyme, 5–15% of the enzyme or enzyme-protein may become enclosed in these structures.[2-4] Liposome-entrapped proteins entered intact cells and insulin-loaded liposomes administered orally caused a drop in blood glucose in diabetic rats.[5] Because factor VIII has been reported to interact hydrophobically with phospholipids when involved in blood coagulation,[6] we thought that it might be possible preferentially to absorb factor VIII on phospholipids and in this way obtain liposomes with a high factor-VIII content in which factor VIII was relatively stable.

Despite problems associated with oral administration of matter contained in liposomes,[7] we thought that because of the specific binding of factor VIII to lipids, administration of factor-VIII-loaded liposomes to a hæmophilic patient might raise the plasma factor VIII content.

METHODS

We prepared factor-VIII-loaded liposomes by shaking a factor VIII solution with glass beads in a flask, the wall of which had been coated with phospholipids. A 200 ml round-bottomed flask was coated with 250 mg of egg lecithin containing 5% (w/w) of phosphatidic acid, by addition of lecithin in ethanol and evaporation of the solution to dryness in a rotary evaporator under reduced pressure. Then 5 ml of the factor VIII solution and a few glass beads were added and the flask was shaken gently until all lipid was removed from the flask wall.

The suspension obtained was centrifuged for 30 min at 27 000 *g* at 10°C which caused the liposomes to float. The aqueous phase was taken out with a hypodermic syringe and used to make a second liposome preparation as described above. Both liposome preparations were pooled and washed once with isotonic saline followed by centrifugation at 50 000 *g* for 10 min. The liposomes were pelleted and diluted to a volume of 50 ml with isotonic saline before oral administration. A more detailed description of the method has appeared elsewhere.[8]

We used factor VIII prepared by cryoprecipitation (AHF Konzentrat SRK [human], Zentral Lab. Blutspendedienst SRK, Switzerland). The freeze-dried material in each ampoule was dissolved in 8 ml of twice-distilled water, and this yielded a preparation containing 7·5 mg/ml of fibrinogen and 43–45 units of factor VIII per ml (one unit of factor VIII is defined as the amount contained in 1 ml of fresh normal plasma).

The supernatant of the saline wash was combined with the infranatant fluid obtained after the second entrapment and factor VIII and fibrinogen were determined. It seemed that this fluid contained 19–22% of the original factor VIII and 76–79% of the fibrinogen. This shows that the liposome preparation is indeed preferentially enriched with factor VIII, probably because of interaction between factor VIII and the lipid bilayer shells.

RESULTS

Plasma factor-VIII activity rose on all three occasions when a liposome preparation of factor VIII was given before breakfast to a patient with severe hæmophilia (mean factor VIII level when not on treatment less than 0·5% of normal; no circulating antibodies). The results in one of the three experiments are shown in the accompanying figure. Plasma concentrations after the same amount of factor VIII had been administered intravenously are also shown. The patient had hæmaturia before ingestion of the liposome preparation; this disappeared on the day of the experiment and returned on the third day after the experiment. Plasma factor-VIII activity did not rise when either liposomes or factor VIII were administered separately.

Some of the factor VIII administered in the liposomes appeared in the plasma as factor-VIII activity. It took